

DP Barcode: D305528

MRID No: 462958-04

**DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)**

1. **CHEMICAL:** Bardac 22C50 (Carboquat) **PC Code No.:** 069208
2. **TEST MATERIAL:** Carboquat **Purity:** 49.85%
Batch No.: 5628-137
3. **CITATION**

Authors: Debbie Desjardins, B.S.
Jon A. MacGregor, B.S.
Henry O. Krueger, Ph.D.
Title: A 96-Hour Toxicity Test of Bardac 22C50 with the Marine
Diatom (*Skeletonema costatum*)
Study Completion Date: May 13, 2004
Laboratory: Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601
Sponsor: Lonza Inc.
17-17 Route 208
Fair Lawn, New Jersey 07410
Laboratory Report ID: 289A-161
DP Barcode: D305528
MRID No.: 462958-04
4. **REVIEWED BY:** Kathryn V. Montague, Biologist **US EPA/AD/RASSB**

Signature:

Date:
5. **APPROVED BY:** Siroos Mostaghimi, Team Leader **US EPA/AD/RASSB**

Signature:

Date:
6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Skeletonema costatum*
Definitive Test Duration: 96 Hours



Type of Concentrations:

Day 0 Measured (14, 20, 26, 40, and 62 Φ g a.i./L)

7. **CONCLUSIONS**

Results Synopsis (Cell Density)

96-hr:

EC₅₀: 25 Φ g a.i./L 95% C.I.: 24 and 26 Φ g a.i./L

NOAEC: 20 Φ g a.i./L

72-hr:

EC₅₀: 23 Φ g a.i./L 95% C.I.: 18 and 30 Φ g a.i./L

48-hr:

EC₅₀: 24 Φ g a.i./L 95% C.I.: 17 and 27 Φ g a.i./L

24-hr:

EC₅₀: 50 Φ g a.i./L 95% C.I.: 44 and 56 Φ g a.i./L

8. **ADEQUACY OF THE STUDY**

A. **Classification:** Acceptable (Core)

B. **Rationale:** No significant deviations from Guideline requirements.

C. **Repairability:** N/A

9. **GUIDELINE DEVIATIONS**

The following guideline deviations were based on EPA OPPTS Guideline 850.5400 (EPA 712-C-96-164):

§ The pH ranged from 8.1 at test initiation to 8.5 at exposure termination. For tests conducted with *Skeletonema costatum*, the guideline requires a pH of 8.1 \forall 0.1. The Study

Report stated that it is typical for increases in pH to occur during tests conducted with *Skeletonema costatum*, due to increases in algal densities.

- \$ The test flasks were shaken continuously at 100 cycles/min versus the guideline requirement of 60 cycles/min for *Skeletonema costatum*.
- \$ The standard deviation was not calculated or plotted and the goodness-of-fit was not determined.
- \$ The following information was not reported:
 - Age of stock culture
 - How much photosynthetically active radiation test chambers received
 - Results and methods for range-finding test
 - If the laboratory periodically runs positive controls with zinc chloride as a reference chemical to ensure that the test algae are responding to a known chemical in an expected manner
 - The maximum labeled rate of the test substance
 - If the analytical method was verified.

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. **Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> \$ <i>Selenastrum capricornatum</i> (<i>Raphidocelis subcapitata</i>) \$ <i>Skeletonema costatum</i> \$ <i>Anabaena flos-aquae</i> \$ <i>Navicula pelliculosa</i>	\$ <i>Skeletonema costatum</i>
<u>Initial Number of Cells</u> \$ 10,000 cells/mL (<i>Selenastrum</i> , <i>Anabaena</i> , <i>Navicula</i>) \$ 77,000 cells/mL (<i>Skeletonema</i>)	\$ 77,000 cells/mL (p.10)
<u>Stock Culture</u> \$ 3 to 7 days old	\$ Age of stock culture not reported \$ Algal cells cultured for at least 14 days prior to

Guideline Criteria	Reported Information
	testing (p.11)
<u>Nutrients</u> \$ Standard formula (ASTM E1218-20) \$ pH 7.5 ∇ 0.1 (<i>Selenastrum</i> , <i>Navicula</i> , <i>Anabaena</i>), 8.1 ∇ 0.1 (<i>Skeletonema</i>) \$ Freshly prepared	\$ Nutrient solutions were prepared by adding reagent-grade chemicals to purified Wildlife International, Ltd. well water. (p.11) \$ pH = 8.1 (p.11) \$ Yes

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u> \$ Upper limit - 0.5 mL/L	\$ No solvent used
<u>Temperature</u> \$ 24E ∇ 2EC (<i>Selenastrum</i> , <i>Navicula</i> , <i>Anabaena</i>) \$ 20E ∇ 2EC (<i>Skeletonema</i>) \$ Recorded hourly	\$ Test chambers were held in an environmental chamber in which the temperature was set to 20 ∇ 2EC. The temperature of a container of water set adjacent to the test chambers in the environmental chamber was recorded twice daily. Water temperatures recorded using a hand held thermometer ranged from 19.8 EC to 21EC . The minimum and maximum temperatures measured continuously throughout the study were 18 and 20 EC. (p.17) \$ Air temperature (which is more likely to fluctuate than solution temperature) was measured continuously using a Fulscope Recorder and a min/max thermometer.
<u>Light Intensity</u> \$ 4.3 K lx (∇ 10%) (<i>Selenastrum</i> , <i>Skeletonema</i> , <i>Navicula</i>) \$ 2.2 K lx (∇ 10%) (<i>Anabaena</i>)	\$ 4210 to 4870 lux (p.17)

Guideline Criteria	Reported Information
\$ Photosynthetically active radiation approx. 66.5 ∇ 10% $\Phi E_{in}/m^2/sec$	\$ Not Reported
<u>Photoperiod</u> \$ 14-hr light/10-hr dark (<i>Skeletonema</i>) \$ Continuous (<i>Selenastrum</i> , <i>Navicula</i> , <i>Anabaena</i>)	\$ 14-hr light/10-hr dark (p.12)
<u>pH</u> \$ 7.5 ∇ 0.1 (<i>Selenastrum</i> , <i>Navicula</i> , <i>Anabaema</i>) \$ 8.1 ∇ 0.1 (<i>Skeletonema</i>) \$ Measured at beginning and end of test	\$ The pH ranged from 8.1 at test initiation to 8.5 at exposure termination. The study author states that the pH was believed to have increased relative to increases in algal densities, typical for tests conducted with <i>Skeletonema costatum</i> . (p.17) \$ Yes (p.12)
<u>Oscillation Rates</u> \$ 100 cycles/min (<i>Selenastrum</i>) \$ 60 cycles/min (<i>Skeletonema</i>)	\$ The test flasks were shaken continuously at 100 rpm. (p.12)
<u>Test Containers</u> \$ 125-500 mL Erlenmeyer flasks \$ Cleaned/sterilized (solvent and acid) and conditioned \$ Test solution volume # 50% of flask volume	\$ 250-mL Erlenmyer flasks (p.12) \$ Sterilized and pretreated for 24 hours with Bardac 22C50 solution of each respective treatment (p.12) \$ Each flask contained 100 mL of test solution or control medium. (p.12)
<u>Dilution Water</u> \$ Sufficient quality (e.g., ASTM Type I) \$ Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	\$ Instant artificial saltwater with a salinity of 30 ppt. (p.11)

C. Test Design

Guideline Criteria	Reported Information
<u>Range-Finding Test</u> \$ Water solubility and physical-chemical properties of test chemical determined? \$ Validated analytical method developed? \$ Expose algae to widely spaced (e.g., log interval) chemical concentration series \$ Lowest value should be at detection limit \$ Upper value, for water soluble compounds, should be at saturation concentration \$ Minimum of 3 replicates \$ Algae should be exposed for 96 hours \$ If highest concentration (saturation concentration or 100 mg/L) results in <50% reduction in growth, a definitive test may not be necessary \$ If lowest concentration (detection limit) results in >50% reduction, definitive test necessary	\$ An exploratory range-finding test was conducted to determine the nominal test concentrations; however, the methods and results of the range-finding test were not reported. (p.10) \$ An analytical method was developed by Wildlife International, Ltd. No information was provided on the validation of the method. (p.13)
<u>Dose Range</u> \$ 1.5X -2X progression	\$ 1.5X progression
<u>Doses</u> \$ 5 or more concentrations of test substance in a geometric series \$ > 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC ₅₀	\$ Geometric series of 5 concentrations. Nominal concentrations were 16, 24, 37, 55, and 83 Φ g a.i./L. Day 0 measured concentrations were 14, 20, 26, 40, and 62 Φ g a.i./L. \$ 97% inhibition of growth at the highest concentration. (p.17)
<u>Controls</u> \$ Negative and/or solvent each test \$ Positive - zinc chloride (periodically)	\$ Negative control \$ Not Reported

Guideline Criteria	Reported Information
<u>Replicates Per Dose</u> \$ 3 or more (4 or more for <i>Navicula</i>)	\$ 3 replicates (p.10)
<u>Duration of Test</u> \$ 96-hr	\$ 96-hr
<u>Growth</u> \$ Logarithmic growth (controls) by 96-hr or repeat test (increase by a factor of 16) \$ 1.5×10^6 cells/mL (<i>Skeletonema</i>) \$ 3.5×10^6 cells/mL (<i>Selenastrum</i>)	\$ The mean cell density in the control was 3.6×10^6 cells/mL by 96 hours. (p.24)
Daily Observations?	Yes (p.14)
<u>Method of Observations</u> \$ Direct - microscopic cell count of at least 400 cells/flask \$ Indirect - spectrophotometry, electronic cell counter, dry weight, etc.; calibrated by microscopic count \$ Qualitative and descriptive	\$ Cell counts were performed using an electronic particle counter. (p.15)
<u>Cell Separation</u> \$ Syringe, ultrasonic bath, or blender; limited sonification (<i>Anabaena</i>) \$ Manual or rotary shaking only (<i>Selenastrum</i> , <i>Skeletonema</i> , <i>Navicula</i>)	\$ The test flasks were shaken continuously at 100 rpm. (p.12)
Algistatic and algicidal effects differentiated?	Yes, based on the cell density observed at the end of the recovery phase, the effect on algal growth at the highest concentration was found to be algistatic. (p.18)
Maximum Labeled Rate	Not Reported

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in the report?	Yes
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	Yes, parent stock of <i>Skeletonema costatum</i> was obtained from Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) and had been maintained in culture medium at Wildlife International, Ltd., Easton, Maryland. (p.11)
Growth in controls reported?	Yes
Description of test system and test design included?	Yes
Initial and final chemical concentrations and pH measured?	Yes (p.12)
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	Yes (p.24)
96-hr EC ₅₀ and when sufficient data generated 24-, 48-, and 72-hr EC ₅₀ , and 95% C.I. reported?	Yes (p.25)
Raw data included?	Yes (p.46)
Methods and data records reported?	Yes
Statistical Analysis \$ Mean and standard deviation calculated and plotted? \$ Goodness-of-fit determined?	\$ Standard deviation not calculated or plotted \$ No

Dose Response at 96-hr

Table 1. Cell Density Values for <i>Skeletonema costatum</i> After 24-, 48-, 72-, and 96-hour Exposure to Bardac 22C50					
Day 0 Measured Test Concentration (Φg a.i./L)	Cell Density (cells/mL) ^a				
	24-hr	48-hr	72-hr	96-hr	% Inhibition ^b
Negative Control	333,510	1,007,116	2,784,369	3,643,489	--
14	318,558	925,405	2,648,126	3,649,455	-0.16
20	270,913	632,265	1,648,951	3,594,662	1.3
26	268,915	444,787	779,946	1,295,015*	64
40	197,907	405,859	860,160	1,594,933*	56
62	133,399	143,394	152,609	125,858*	97

^a Mean value of three replicates^b Percent inhibition compared to the negative control replicates at 96 hours* Statistically significant difference ($p < 0.05$) at 96 hours from the negative control replicates using Dunnett=s test, as determined by the study author.**Statistical Results**

Statistical Method: Non-linear regression or linear interpolation was used to calculate EC₅₀ values and their corresponding 95% confidence intervals for cell density (EC₅₀) for each 24-hour exposure period, when possible. The 96-hour NOAEC was calculated by evaluating the data for normality and homogeneity of variance ($p = 0.05$) using the Shapiro-Wilk=s and Levene=s tests, respectively. The treatment groups were then compared to the negative control using Dunnett=s Test ($p = 0.05$).

The Day 0 measured test concentrations were used to calculate the EC₅₀ and NOAEC values. The Day 0 measured test concentrations ranged from 71 to 87% of nominal concentrations and the Day 4 measured test concentrations ranged from 26 to 57% of nominal concentrations.

96-hr (Cell Density):

EC₅₀: 25 Φ g a.i./L 95% C.I.: 24 and 26 Φ g a.i./L
NOAEC: 20 Φ g a.i./L

72-hr (Cell Density):

EC₅₀: 23 Φ g a.i./L 95% C.I.: 18 and 30 Φ g a.i./L

48-hr (Cell Density):

EC₅₀: 24 Φ g a.i./L 95% C.I.: 17 and 27 Φ g a.i./L

24-hr (Cell Density):

EC₅₀: 50 Φ g a.i./L 95% C.I.: 44 and 56 Φ g a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Results were verified using ANOVA with Dunnett's Test (TOXSTAT) for the NOAEC, and linear regression (TOXANAL) for the EC₅₀ value. Results were in agreement with reported results.

14. REVIEWER=S COMMENTS:

§ Guideline deviations are noted in Section 9.

Sign-off Date : 11/04/05
DP Barcode No. : D305528